Targeted-Venom Discovery Array™ assists in identifying GPCR agonists

Venom peptide libraries deliver novel hits for challenging targets

TOP SPECS

Number of fractions	956	Novelty factor (1 / published papers)	1
Hit rate	2.5%	Number of hits	24
Z' of assay	0.697	Hit potency	0.55 µM
Targeting level 1-5 (1 = general, 5 = specific)	3	Diversity	3

Free fatty acids (FFAs) are known nutrients, but they also participate in physiological processes via FFA receptors such as FFAR4, otherwise known as the G-protein coupled receptor 120 (GPR120). GPR120 stimulates incretin hormone release from colonic endocrine cells and is implicated in macrophage and adipocyte function. Activation of GPR120 has been linked with inhibition of inflammation, modulation of hormone secretion from the pancreas and gastrointestinal tract, and the regulation of lipid and/or glucose metabolism in adipose, liver, and muscle tissues. GPR120 agonism correlates well with stabilizing metabolic homeostasis and consequently, the prevention and development of metabolic disorders such as obesity and diabetes. As a result, GPR120 has been identified as a potential target for drug discovery. However, the natural ligands for this receptor – FFAs – are not ideal contenders for drug development. Previous attempts to identify agonists from traditional compound libraries have not been successful, making this a challenging receptor for drug discovery and novel drug candidates.

Venom peptides serve as perfect alternatives as they have evolved naturally to act as ligands for a range of receptors and ion channels in predators and prey. Since they are secreted into the lumen of the venom gland and may remain there for extended periods of time ready for rapid delivery in under a second, they are naturally exceptionally stable molecules. Venomtech's venom peptide libraries – known as Targeted-Venom Discovery Array™ libraries – make use of these properties to help in understanding specific interactions between receptors and their ligands, as well as the development of novel therapeutics.

This technical note describes the use of targeted venom peptide libraries and chemiluminescent detection of the interaction of β-arrestin with activated GPCRs to identify peptides with agonistic effects for GPR120.[1]

Figure 1: Method overview.

METHOD

A Targeted-Venom Discovery Array^{GPCR} (T-VDA^{GPCR}) containing 956 venom fractions was engineered from venoms extracted from nine elapid snakes, four pit vipers and a lizard. All venoms were two-dimensionally fractionated using an UltiMate™ 3000 UHPLC system (Thermo Scientific), and each T-VDA^{GPCR} was standardised, assembled, and lyophilised in Echo®-qualified, 384-well plates. PathHunter® eXpress GPR120S CHO-K1 β-Arrestin GPCR Assay Kits (DiscoverX) were screened to identify peptides with agonistic effects against GPR120. The pre-validated, positively expressing cells were plated in 384-well cell culture plates (10,000 cells/well) according to the assay protocol and then incubated for 24 hours at 37 °C and 5 % CO2. The T-VDA^{GPCR} fractions were dissolved in 15 µl assay cell culture media per well (13.3 µg/ml), then 2.5 µl aliquots of each fraction were transferred to each well of the cell culture plates to give a final working concentration of 1.2 µg/ml, before incubating for 90 minutes at 37 °C and 5 % CO2 (N=2). Following this, 13.8 µl of complete substrate reagent was added to all wells and the plates were incubated in the dark for 60 minutes.

Luminescence was measured at room temperature every 30 minutes up to two hours post-incubation, using a CLARIOstar® Plus plate reader (BMG LABTECH) and quick luminescence settings. Data was analysed as relative luminescence, and the most promising hits were followed up in concentration-response mode. Concentration-response curves (CRCs) were produced in triplicate according to the protocol above, diluting the hit fractions to final working concentrations of 100, 50, 33, 16.5, 10, 5, 3.3, 1.65, 1 and 0.33 µg/ml, where stocks allowed. Luminescence readings were taken and analysed as before and plotted as log concentration vs response. Finally, selected hits were identified by intact mass analysis and peptide mapping (trypsin/chymotrypsin double digest) mass spectrometry.

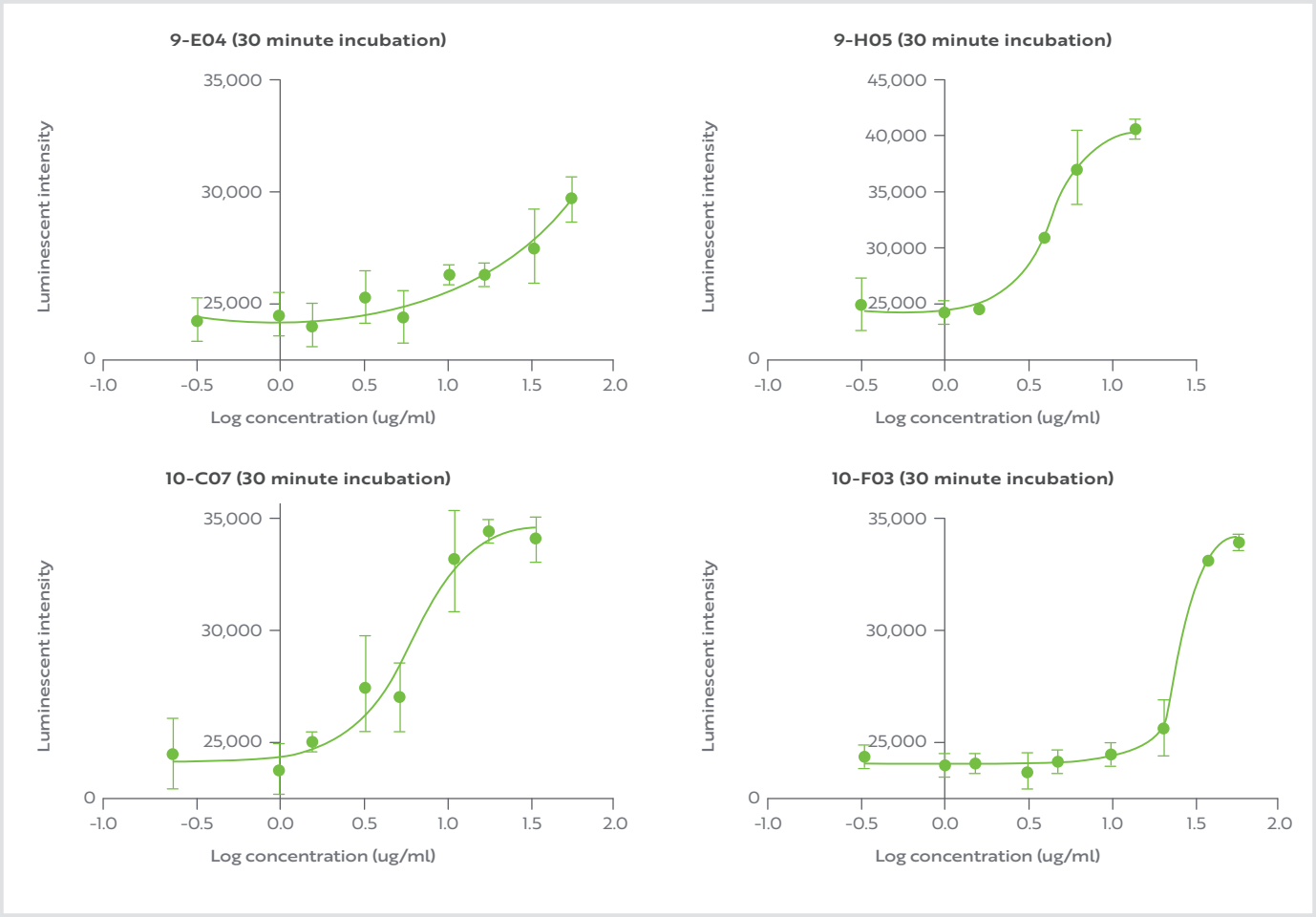

Figure 2: Agonism of GPR120 by venom fractions demonstrated by concentration-response curves. EC_{50} values: 9-E04 - 4.098 mM (27 mg/ml), 9-H05 - 0.559 µM (3. 75 ug/ml), 10-C07 - 0.81 µM (5.5 ug/ml), and 10-F03 – 3.176 µM (21.4 ug/ml).

RESULTS

A mini Z' analysis confirmed the expected assay robustness (Z' = 0.697) and 24 hit fractions were initially identified (2.5 % hit rate). Of these, 18 fractions from elapid snakes and one viper venom fraction were selected for follow-up with CRCs.

The four most potent hits – all from the venom of *Naja siamensis* – were selected for MS identification. The CRCs (Figure 2) display the ability and varying potency of these fractions to elicit complete or partial agonism of GPCR120. Mass spectrometry identified the hits as cobra three-finger cytotoxins. Venom peptide 9-E04 was a 98.3 % match to Cytotoxin SP15d from *Naja atra*, 9-H05 a 90 % match to Cytotoxin Vc-5 from *Naja oxiana*, and 10-C07and10-F03 were 95 % and 88.3 % matches, respectively, for two peptides, Cytotoxin10 from *Naja annulifera* and Cytotoxin 2 from *Naja nivea*. Sequence analysis identified that these cytotoxins have a highly conserved structure, sharing a 68.3 % exact sequence match across all four peptides. 19 amino acids are variable amongst the four sequences and are therefore the key residues responsible for the differences observed in potency.

CONCLUSIONS

Venomtech's range of T-VDA™ solutions is purified and freeze-dried from carefully selected venomous species and specifically engineered to maximise the chance of success and deliver the greatest value in the generation of fresh leads. Each T-VDA™ is supplied in Echo®-qualified, 384-well plates for high throughput, assay-ready plug-and-play convenience.

This technical note demonstrates that venom peptide libraries can deliver novel hits when screened against challenging targets such as GPCR120. It shows that GPCR receptors can be modulated by venom peptides, offering an alternative to FFAs. In addition, it provides evidence that three-finger toxins can act as agonists for novel GPCR receptors, and this property could be further applied to the drug design of smaller peptides. The discovery of novel venom actions is leading to a better understanding of the ligand-receptor interactions of these peptides in nature, and the toxins themselves would make excellent tools for GPCR research.

[1] Novel GPCR ligands: GPR120 case study. McCullough, D., Baker, S and Grant, P. ELRIG Drug Discovery 2021.

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